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DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

EXAMINER	
SWOPE, SHERIDAN	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/573,821	OKUNO ET AL.	
	Examiner	Art Unit	
	SHERIDAN SWOPE	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 September 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28-MAR-2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *0709;0909*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' filing of September 14, 2009, in response to the action mailed May 14, 2009, is acknowledged. It is acknowledged that Claims 1-35 have been amended. Claims 1-35 are pending.

Based on the petition decision of October 13, 2009, the lack of unity of February 9, 2009 is withdrawn. The action of May 14, 2009 is also withdrawn and replaced with the action herein. Claims 1-35 are hereby considered.

The examined invention is directed to a method for cleavage using E. coli OmpT protease or a variant thereof having a substitution of Asp^{97} with Ala, Leu, Phe, Met, Ser, Thr, Cys, Asn, Gln, Glu, or His.

Priority

For the instant invention, priority is granted to PCT/JP04/14704, filed September 29, 2004, which disclosed the elected invention. The examiner cannot consider whether JP 2003-342183, filed September 20, 2003, disclosed the recited invention because an English translation thereof has not been made available by applicants.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Double Patenting

> The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

An obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but an examined application claim not is patentably distinct from the reference claim(s) because the examined claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985).

Claims 1, 4-7, 34, and 35 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-3, 12, and 19 of US Patent 6,037,145. Although the conflicting claims are not identical, they are not patentably distinct from each other. Claims 1, 4-7, 34, and 35 herein and Claims 1-3, 12, and 19 of US 6,037,145 are both directed to methods for cleaving a polypeptide using OmpT protease, wherein the polypeptide comprises a P1 Arg and one, two, or three basic amino acids within P3-P10, wherein the single basic amino acid is not at P4. The claims differ in that Claims 1-3, 12, and 19 of US 6,037,145 specifically recite cleavage of a fusion protein, while Claims 1, 4-7, 34, and 35 herein recite cleavage of any protein. The portion of the specification in US 6,037,145 that supports the recited methods includes embodiments that would anticipate Claims 1, 4-7, 34, and 35 herein, e.g., methods for cleaving fusion proteins using an OmpT protease, which are also the methods specifically recited in Claims 1-3, 12, and 19 of US 6,037,145. Claims 1, 4-7, 34, and 35 herein cannot be considered patentably distinct over Claims 1-3, 12, and 19 of US 6,037,145 when there are specifically recited embodiments (methods for cleaving fusion proteins using an OmpT protease) that would anticipate Claims 1, 4-7, 34, and 35 herein. Alternatively, Claims 1, 4-7, 34, and 35 herein cannot be considered patentably distinct over Claims 1-3, 12, and 19 of US 6,037,145 when there are specifically disclosed embodiments in US 6,037,145 that supports

Claims 1-3, 12, and 19 of that patent and falls within the scope of Claims 1, 4-7, 34, and 35 herein, because it would have been obvious to a skilled artisan to modify the methods of Claims 1-3, 12, and 19 of US 6,037,145 by selecting a specifically disclosed embodiment that supports those claims, i.e., methods for cleaving fusion proteins using an OmpT protease, as disclosed in US 6,037,145. One having ordinary skill in the art would have been motivated to do this, because such an embodiment is disclosed as being a preferred embodiment within Claims 1-3, 12, and 19 of the other patent.

Claim Rejections - 35 USC § 112-Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-35 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the following reasons.

For Claim 1-3, 8, 12, 15, 16, 18, 23, 27-30, 34, and 35, the phrase "E. coli OmpT protease" renders the claim indefinite because neither the specification nor the prior art specifically define the structure or function of the genus of said encompassed E. coli OmpT proteases. Regarding the functional limitations, the specification states:

> "Sugimura et al. have examined the substrate specificity of OmpT protease and have reported that the enzyme specifically cleaves the central peptide bonds between the basic amino acid pairs of arginine-arginine, lysine-lysine, arginine-lysine and lysine-arginine (Sugimura, K. and Nishihara, T. J. Bacteriol. 170: 5625-5632, 1988)." [0003]
>
> "However, the enzyme does not cleave all basic amino acid pairs, as it is highly specific. For example, human γ-interferon contains 10 basic amino acid pairs, but only two of them are cleaved (Sugimura, K. and Higashi, N.J. Bacteriol. 170: 3650-3654, 1988). This is attributed to the influence of the three-dimensional structure of the human γ-interferon substrate and to the amino acid sequences of sites thought to be recognized by the enzyme which are adjacent to basic amino acid pairs." [0004]

> "OmpT protease cleavage sites have been discovered with amino acid sequences other than basic amino acid pairs, and Dekker et al., using substrates with amino acid substitutions introduced into an OmpT protease substrate comprising the amino acid sequence Ala-Arg-Arg-Ala (P2-P1↓P1'-P2'), have reported that OmpT protease exhibits high specificity for the basic amino acids arginine and lysine as the amino acid at the P1 position of the cleavage site, but is less stringent in regard to the amino acid at the P1' position (Dekker, N. et al. Biochemistry 40: 1694-1701, 2001)." [0008]
>
> "As for the specificity with respect to the sequences adjacent to the cleavage site, it has been demonstrated that cleavage fails to occur when an acidic amino acid is present at the P2 or P2' position (Dekker, N. et al. Biochemistry 40: 1694-1701, 2001)." [0010]

These statements indicate that, at the time of filing, there was confusion in the art as to the substrate specificity, i.e., functional limitations, for the genus of E. coli OmpT proteases. While, the E. coli OmpT protease of Sugimura et al was known to cleave between dibasic residues, not all dibasic residues were cleaved. Moreover, the art taught that unknown properties of a polypeptide, including residues other than P2, P1, P1', and P2' as well as the three dimensional structure, affect the ability of the polypeptide to be cleaved by the E. coli OmpT protease of Sugimura et al. Thus, at the time of filing, the functional metes and bounds for the E. coli OmpT protease of Sugimura et al, as well as any other E. coli OmpT proteases, were unclear. Moreover, the structural limitations for the genus of E. coli OmpT proteases were also unclear. A BLAST search with the E. coli OmpT protease of Sugimura et al, 1988a (spec [0003]; AAA24430.1) disclosed proteins annotated as E. coli OmpT proteases and having as little as 70% identity with Sugimura's protein (for example, YP_444072.1; enclosed). It is unclear whether such proteins are encompassed by the recited genus. Thus, the skilled artisan would not know the genus encompassed by the phrase "E. coli OmpT protease" because neither the functional nor structural metes and bounds are distinctly described. Claims 4-7, 9-11, 13, 14, 17, 19-22, 24-26, and 31-33, as dependent from Claim 1-3, 8, 12, 15, 16, 18, 23, 27-30, 34, and/or 35, are indefinite for the same reason. For purposes of examination, it is assumed that "E.

coli OmpT protease" means the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1) and is herein referred to as "E. coli OmpT protease" or "OmpT protease".

Regarding the prior rejection of Claims 12, 15-16, 23, and 26-27 for the phrase "OmpT protease", Applicants argue that said phrase is defined on pages 7-8 of the specification. It is acknowledged that the paragraph bridging pages 7-8 states:

> "According to the invention, "OmpT protease" refers to mature OmpT protease from E. coli after removal of the signal peptide, or a protein other than OmpT protease having OmpT protease activity (OmpT-like protease). As OmpT-like proteases there may be mentioned (I) Yersinia pestis plasminogen activator, (2) Salmonella typhimurium E protein, (3) Escherichia coli and (4) Shigella flexneri SopA."

Said paragraph fails to define (i) the specific function encompassed by "OmpT protease activity" or (ii) the structure or function encompassed by the genus of "OmpT-like protease" proteins. Moreover, the claims, as amended, recite "E. coli OmpT protease"; see rejection above.

For Claims 1-35, the phrase "desired cleavage site" renders the claim indefinite. It is acknowledged that the specification states:

> "The term "desired cleavage site" according to the invention refers to any site in the polypeptide, a site between the C-terminus of the linker peptide of the fusion protein composed of the target protein fused with the protected peptide via the linker peptide, and the N-terminus of the target peptide, or any site in the linker-peptide." [0040]

However, said statement fails to define the number of amino acids positions within a "desired cleavage site". Thus, it is unclear whether the "desired cleavage site" consists of only P1-P1' (two positions), P10-P5' (15 positions), or any other number of residues. The skilled artisan would not know the metes and bounds of the recited invention. For purposes of examination, it is assumed that the "desired cleavage site" consists of between P1-P1' (two positions) and P10-P5' (15 positions).

For Claim 2, the phrase "wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the protecting peptide is the P1' position" renders the claim indefinite. Since the P1' position is C-terminal to the P1 position (P2-P1↓P1'-P2'), the protecting peptide cannot have both a P1 C-terminus and a P1' N-terminus. The skilled artisan would not know the metes and bounds of the recited invention. Claims 31-33, as dependent from Claim 2, are rejected for the same reason. For purposes of examination, it is assumed that said phrase is meant to say "wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the target peptide is the P1' position", i.e., the protecting peptide is X-X-P1-C- fused directly to the –N-P1'-X-X- target peptide. Said assumption is supported, for example, by original Claim 2.

For Claims 8 and 23, the phrase "producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein" renders the claim indefinite. A peptide cannot comprise an action, i.e., activity. It is unclear whether said phrase means cleaving the fusion protein at a specific site with OmpT protease or cleavage by some other process. The skilled artisan would not know the metes and bounds of the recited invention. Claim 9, as dependent from Claim 8, is indefinite for the same reason. For purposes of examination, it is assumed that "producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein" means cleaving the fusion protein at a specific desired site with OmpT protease.

For Claims 14 and 17, the phrase "any site" renders the claim indefinite. It is unclear whether said phrase means "any position" or "the desired cleavage site". The skilled artisan would not know the metes and bounds of the recited invention. For purposes of examination, it is assumed that "any site" means "any position".

For Claim 16, the phrase "protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site" renders the claim indefinite. It is unclear whether said phrase means (i) the C-terminal Arg or Lys of the protecting peptide is fused directly to the N-terminal non-Arg or non-Lys of the target peptide and the cleavage site is between said two residues, (ii) the C-terminal Arg or Lys of the protecting peptide is fused, via a linker, to the N-terminal non-Arg or non-Lys of the target peptide and the cleavage site is within said linker, or (iii) (i) and (ii). The skilled artisan would not know the metes and bounds of the recited invention. For purposes of examination, it is assumed that "protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site" means (i) or (ii), above.

For Claim 34, the phrase "using, as the cleaving protease, bacterial cells expressing a gene coding for…" renders the claim indefinite. It is unclear whether said phrase means using intact bacterial cells, a lysate thereof, and/or the isolated encoded protease. The skilled artisan would not know the metes and bounds of the recited invention. For purposes of examination, it is assumed that "using, as the cleaving protease, bacterial cells expressing a gene coding for…" means using intact bacterial cells, a lysate thereof, or the isolated encoded protease.

For Claim 35, the term "co-expressed" renders the claim indefinite. It is unclear whether said term means recombinant co-expression and/or endogenous co-expression. The skilled artisan would not know the metes and bounds of the recited invention. For purposes of examination, it is assumed that "co-expressed" means recombinant co-expression and/or endogenous co-expression of either or both the protease and the substrate protein.

Claims 2, 3, 12, 15-17, 27, 34 and 35, and dependent claims thereof, are rendered indefinite for improper antecedent usage as follows.

For Claims 2 and 3, the phrase "E. coli OmpT protease" should be corrected to "the E. coli OmpT protease".

For Claim 12, 15, 16, 27, 34 and 35, the phrase "wherein the 97th amino acid from the N-terminus of the OmpT protease is…" should be corrected to "wherein the 97th amino acid from the N-terminus of the OmpT protease variant is…".

For Claim 17, the phrase "a desired cleavage site" should be corrected to "the desired cleavage site".

For Claim 17, the phrase "a fusion protein" should be corrected to "the fusion protein".

For Claim 17, "the amino acid sequence" lacks antecedent basis.

For Claim 35, the phrase "a polypeptide" should be corrected to "the polypeptide".

Any subsequent rejection based, on clarification of the above phrases and terms, will not be considered a new ground for rejection.

The following definitions are noted:

> "The term "target peptide" according to the invention is used not only in regard to the peptide which is to be finally obtained, but also including any production intermediate ("precursor peptide") which, after cleavage from the fusion protein by OmpT protease or the like, is subjected to subsequent modification reaction or cleavage reaction." [0038]

> "The term "protecting peptide" according to the invention is used to refer to a peptide which forms a fusion protein with the target peptide via a linker peptide, and it includes the linker peptide." [0039]

Claim Rejections - 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

This rejection is based on the assumption that that "E. coli OmpT protease" means the OmpT protease of Sugimura et al, 1988a (AAA24430.1) and that the recited OmpT Asp^{97} variants are derived from said OmpT protease sequence of Sugimura et al, 1988a. This rejection is also based on the assumption that, as described above, a desired cleavage site may consist of up to 15 amino acid residues, which represents 3.3×10^{19} sequences

Claims 12-30, 32, 34, and 35 are rejected under 35 U.S.C. 112, first paragraph/enablement for the following reasons. The art is enabling for cleaving essentially any OmpT protease substrate with the Asp97Glu OmpT protease variant because the skilled artisan would believe that, more likely than not, said variant has the same substrate specificity as the parent protease. In addition, the specification is enabling for using:

- Asp^{97}Met OmpT to cleave the sites AAR↓RR↓AR↓FVPIF, ADR↓RR↓AR↓FVPIF, and DARRR↓AR↓FVPIF (Fig5,15), and to minimally cleave LRLYR↓[A/V/F/S/C/Y]HHGS (Exmp13;Table 1)
- Asp^{97}Leu OmpT to cleave the site RAR↓SYSME (Fig11-12), and to minimally cleave the sites LRLYR↓[A/F/S/C/Y]HHGS (Exmp13&Table 1)
- Asp^{97}His OmpT to cleave the sites AAR↓RR↓AR↓CGNLS (Fig11-12) and to minimally cleave the sites LRLYR↓[A/V/I/F/M/S/T/C/N/K/R]HHGS (Exmp13;Table 1)
- Asp^{97}Ala OmpT to minimally cleave the site LRLYR↓[A]HHGS (Exmp13;Table 1)
- Asp^{97}Phe OmpT to minimally cleave the site LRLYR↓[A]HHGS (Exmp13;Table 1)
- Asp^{97}Ser OmpT to minimally cleave the sites LRLYR↓[A/C]HHGS (Exmp13;Table 1)
- Asp^{97}Thr OmpT to minimally cleave the sites LRLYR↓[A/V/F/S/C/K/R]HHGS (Exm13;Table 1)
- Asp^{97}Gln OmpT to minimally cleave the sites LRLYR↓[A/S]HHGS (Exmp13;Table 1)
- Asp^{97}Asn OmpT to minimally cleave the sites LRLYR↓[A/S/C]HHGS (Exmp13;Table 1)

However, the specification does not reasonably provide enablement for cleaving any site with an OmpT protease having any substitution with Ala, Leu, Phe, Met, Ser, Thr, Cys, Asn, Gln, or His at Asp^{97} of OmpT protease. The specification does not enable any person skilled in

the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In regards to this enablement rejection, the application disclosure and claims are compared per the factors indicated in the decision In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). These factors are considered when determining whether there is sufficient evidence to support a description that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. The factors include but are not limited to: (1) the nature of the invention; (2) the breath of the claims; (3) the predictability or unpredictability of the art; (4) the amount of direction or guidance presented; (5) the presence or absence of working examples; (6) the quantity of experimentation necessary; (7) the relative skill of those skilled in the art. Each factor is here addressed on the basis of a comparison of the disclosure, the claims, and the state of the prior art in the assessment of undue experimentation.

Claims 12-30, 32, 34, and 35 are so broad as to encompass the following.

Claims 12, 15, 18, 23, 24, 34, 35 are so broad as to encompass cleaving any site using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97} of OmpT protease.

Claims 13 and 16 are so broad as to encompass cleaving any site, having a P1 Arg or Lys and not having a P1' Arg or Lys, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97} of OmpT protease.

Claims 14, 17, 21, and 22 are so broad as to encompass cleaving any site, having a P1 Arg or Lys, not having a P1' Arg or Lys, and having a single basic amino acid or two or three consecutive basic amino acids situated at any site in the amino acid sequence from the P10

position to the P3 position or from the P3' position to the P5' position, using an OmpT protease having any substitution of A, L, F, M, S, T, C, Q, N, or H at D^{97} of OmpT protease.

Claims 19 and 20 are so broad as to encompass cleaving any site having two or three consecutive basic amino acids situated at any site in the amino acid sequence from the P10 position to the P3 position, using an OmpT protease having any substitution of A, L, F, M, S, T, C, Q, N, or H at D^{97} of OmpT protease.

Claim 25 is so broad as to encompass cleaving any site, comprising R-R-R-A-R(P1)↓X(P1') wherein P1' is any amino acid, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97} of OmpT protease.

Claim 26 is so broad as to encompass cleaving any site, comprising D-A-R-R-R-A-R(P1)↓X(P1') wherein P1' is any amino acid, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N,Q, or H at D^{97} of OmpT protease.

Claim 27 is so broad as to encompass cleaving any site using an OmpT protease having any substitution of L, M, or H at D^{97} of OmpT protease.

Claim 28 is so broad as to encompass cleaving any site, having a P1' Ser or Arg, using an OmpT protease having a substitution of L at D^{97} of OmpT protease.

Claim 29 is so broad as to encompass cleaving any site, having a P1' F, A, S, C or Y, using an OmpT protease having a substitution of M at D^{97} of OmpT protease.

Claim 30 is so broad as to encompass cleaving any site, having a P1' A, V, I, M, S, T, C, or N, using an OmpT protease having a substitution of H at D^{97} of OmpT protease.

Claim 32 is so broad as to encompass cleaving a protein comprising any target protein having any structure and having the function any calcitonin precursor.

The scope of each of these claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of methods broadly encompassed by the claim. Since the amino acid sequence of a protease determines its structural and functional properties, predictability of which changes can be tolerated in a protease's amino acid sequence and obtain the desired activity to cleave the recited genera of sites requires a knowledge of and guidance with regard to which amino acid substitutions allow cleavage of which sites and detailed knowledge of the ways in which the protease's structure relates to its function to cleave any specific site. However, in this case the disclosure is limited to the specific combinations of OmpT variants and sites listed above.

While methods for making variant proteases and testing said variants for cleavage of specific motifs are known, it is not routine in the art to screen the encompassed ten OmpT variants for cleavage at any site comprising any amino acids at positions P10-P5', as encompassed by the instant claims. Neither the art nor the specification provides sufficient guidance such that the required experimentation would not be undue. The art teaches that Asp^{97} of OmpT protease is critical for P1' cleavage site recognition and that alteration of Asp^{97} causes changes in the efficacy of cleavage (Kramer et al, Fig2-4; pg429, parg1). As explained above under 35 USC 112, second paragraph, neither the specification nor the art defined the substrate specificity such that the skilled artisan would be apprized of the functional metes and bounds of the parent E. coli OmpT protease activity. In addition, while the instant specification teaches that the Asp^{97}Ala OmpT protease variant does not cleave at –Arg-Arg- (Table 1), the art teaches that the Asp^{97}Ala OmpT protease variant does cleave at –Arg-Arg- (Kramer et al, 2001; Fig 2). Thus, cleavage sites for the encompassed OmpT protease variants remained unpredictable.

Moreover, the difference in the results of Kramer et al and the instant specification strongly argue that the amino acid residues at positions P10-P2 and P2'-P5 affect the ability of any peptide motif to be cleaved by the encompassed OmpT protease variants remained unpredictable at the time of filing.

The claims encompass using variant OmpT proteases for cleaving a desired site comprising up to 15 amino acid residues (positions P10-P5'). It is acknowledged that, a P1 Arg is highly favored for cleavage by E. coli OmpT protease (Kramer et al; Fig 4). It is also acknowledged that based on the art and the specification, the skilled artisan would, more likely than not, be able to predict the favored amino acid(s) at the P1' position (in combination with a P1 Arg) for the recited OmpT protease variants (see rejections under 35 USC 103(a)). The art and the specification also teach that acidic amino acids at positions P6 and P4-P2 inhibit cleavage by the E. coli OmpT protease of Sugimura et al (Dekker et al, 2001 and Okuno et al, 2002b; IDS). However, as explained above under 35 USC 112, second paragraph, neither the specification nor the art defined the substrate specificity, encompassing up to positions P10-P5', such that the skilled artisan would be apprized of the functional metes and bounds of E. coli OmpT protease activity. Moreover, no information is provided regarding these positions and cleavage by the encompassed OmpT protease variants. Neither the specification nor the prior art provide evidence as to which amino acid residues are favored, permitted, or non-favored at positions P10-P2 and P2-P5', for cleavage by the recited OmpT protease variants. Thus, cleavage by the recited OmpT protease variants remained unpredictable. The genus of all sites comprising any of the 20 amino acids at said 13 positions is represented by 20^{13} i.e., 8.2×10^{16} sequences, which would need to be tested with the encompassed ten OmpT protease variants.

While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Sufficient guidance has not been provided in the instant specification.

The specification does not support the broad scope of Claims 12-30, 32, 34, and 35, which encompasses the methods outlined above. The specification does not support the broad scope of Claims 12-30, 32, 34, and 35 because the specification does not establish: (A) all amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that support cleavage by any specific Asp^{97} OmpT protease variant; (B) amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that inhibit cleavage by any specific Asp^{97} OmpT protease variant; (C) residues of the desired cleavage motif which may, or may not, be modified without affecting the cleavage activity; (D) the general tolerance of the cleavage activity to modification of any desired cleavage motif and extent of such tolerance; (E) a rational and predictable scheme for modifying any residues in a cleavage motif, in conjunction with modification of the Asp^{97}residue of OmpT protease, with an expectation of obtaining the desired cleavage activity; (F) the structure of all peptides having calcitonin precursor activity (Claim 32); and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any method for cleaving any site with an OmpT protease having any substitution of Ala, Leu, Phe, Met, Ser, Thr, Cys, Asn, Gln, or His at Asp^{97} of OmpT

protease. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the identity of methods having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Regarding the prior rejection of Claims 12, 15-16, 23, and 26-27 under 35 USC 112, first paragraph/enablement, Applicants traverse with the following arguments, which are relevant to the rejection above.

(A) The claims are not as broad as alleged by the Office to encompass infinite methods of cleavage, because both the enzymes and their substrates are specifically defined in the Specification (pg 7-9).

(B) At filing, there was sufficient knowledge in the field regarding the structure-function relationship in OmpT protease. See Kramer et al. 505 FEBS LETT. 426 (2001) (IDS 28-MAR-2006). The crystal structure of OmpT protease has been solved, and the structure, particularly the interaction between the enzymatic active site with the substrate, has provided rational design of OmpT protease variants.

Furthermore, the OmpT protease and other OmpT-like proteases from Salmonella, Yersinia, and Shigella are known to be members of the omptin family. Members of the omptin family share a high level of amino acid sequence identity. See e.g., lines 26-33 on page 1 of the Specification. For example, OmpT protease contains 36 acidic residues, of which six aspartates (positions 43, 83, 85, 97, 208, and 210) and five glutamates (positions 27, 111,136, 193, and

250) are fully conserved within the omptin family. See page 426 and Fig. 1 of Kramer. Thus, knowledge obtained from an OmpT-like protease would be applicable to OmpT protease, e.g., substitutions in the conserved regions are more likely to affect the enzyme's recognition and cleavage of a specific site in a polypeptide.

(C) The present Specification provides working examples in Examples 13-18 in which the OmpT variants with a substitution at the 97th amino acid position are used to cleave polypeptides with various cleavage motifs. The required experimentation would have been considered as routine.

(A) Reply: It is acknowledged that the paragraph bridging pages 7-8 states:

> "According to the invention, "OmpT protease" refers to mature OmpT protease from E. coli after removal of the signal peptide, or a protein other than OmpT protease having OmpT protease activity (OmpT-like protease). As OmpT-like proteases there may be mentioned (I) Yersinia pestis plasminogen activator, (2) Salmonella typhimurium E protein, (3) Escherichia coli and (4) Shigella flexneri SopA."

Said statement fails to define the specific function or structure encompassed by the genus of "E. coli OmpT protease" or the substrates thereof (see rejection under 35 USC 112, second paragraph). Likewise, said pages fail to define the specific function or structure encompassed by the genus of the encompassed E. coli OmpT protease variants. While enablement is not precluded by the necessity for routine screening, as explained above, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Sufficient guidance has not been provided in the instant specification.

(B) Reply: It is acknowledged that the crystal structure of OmpT protease was published by Vandeputte-Rutten et al, 2001 (IDS); however, Vandeputte-Rutten et al fails to teach any Asp^{97} OmpT protease variants or substrates thereof. Only one relevant OmpT protease

variant, $Asp^{97}Ala$, and one substrate thereof was known at the time of filing (Kramer et al, 2001; IDS). However, these results of Kramer et al do not agree with results of this application (Table 1). As explained above, neither the specification nor the prior art provide evidence as to which amino acid residues are favored, permitted, or non-favored at positions P10-P2 and P2-P5', for cleavage by the recited OmpT protease variants. Thus, cleavage by the recited OmpT protease variants remained unpredictable.

It is acknowledged that other OmpT-like proteases have 40-73% homology with OmpT protease (Hritonenko et al, 2007; Table I). However, said homologies fail to provide evidence as to the substrate specificity for variants of OmpT protease with a substitution at Asp^{97}. It is acknowledged that teachings regarding the substrate specificity of OmpT-like proteases with a substitution at Asp^{97} might be applicable to OmpT protease with a substitution at Asp^{97}. However, the specification fails to point to such teachings. Moreover, the art teaches that E. coli OmpP protease, which has highest identity with OmpT protease has a different substrate specificity (Hwang et al, 2007). Thus, the art teaches away from using other omptin proteases as models for the substrate specificity of Asp^{97} OmpT protease variants.

(C) Reply: It is acknowledged that Examples 13-18 provide working examples in which OmpT Asp^{97} variants are used to cleave some cleavage motifs. However, for the reasons explained above said examples do not enable the skilled artisan to (i) cleave any site using an OmpT protease variant having any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97}, (ii) cleave any site having any amino acid at positions P10-P2 and P2'-P5' using a sub-genus of OmpT protease variants having a substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97}, or (iii) cleave any site having any amino acid at positions P10-P7, P5, and P2'-P5', wherein P2-P4 and

P6 are not acidic amino acids, using a sub-genus OmpT protease variants having a substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97}.

For these reasons and those explained above and in the prior action, Claims 12-30, 32, 34, and 35 are rejected under 35 U.S.C. 112, first paragraph/enablement.

Written Description

These rejections are based on the assumption that that (i) "E. coli OmpT protease" means the OmpT protease of Sugimura et al, 1988a (AAA24430.1), (ii) the recited OmpT Asp^{97} variants are derived from said OmpT protease sequence of Sugimura et al, 1988a, and (iii a desired cleavage site may consist of up to 15 amino acid residues, which represents 3.3×10^{19} sequences

Claims 1-3, 6, 7, and 31-35 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide, wherein the polypeptide has a cleavage motif comprising a basic residue at P1, a P1' that is not Asp, Glu, or Pro, and a single basic amino acid residue between P3-P10, P3'-P5', or P3-P5, but not at P4 or P4', wherein the polypeptide is cleaved at said motif by OmpT protease. The specification teaches several representative species of such methods wherein the P1' position is Arg (Fig2) but teaches no representative species of such methods wherein the P1' position is not Arg. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by OmpT protease. As explained

above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for E. coli OmpT protease, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 1-7, 10, 11, and 31-35 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide with OmpT protease, wherein the polypeptide has a cleavage motif comprising a basic residue at P1, a P1' that is not Asp, Glu, or Pro, and two or three consecutive basic amino acids residues between P3-P10, P3'-P5', or P3-P5. The specification teaches only a single representative species of such methods; wherein the motif comprises Arg at P1 and P3-P5 and Ser at P1' (Fig11-12). Moreover, the specification fails to describe any other representative motifs by any identifying characteristics or properties other than the functionality of being a site for cleaving a polypeptide by OmpT protease. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for E. coli OmpT protease, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 12, 15, 18, 23, 24, 34, 35 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, E, or H at D^{97} of OmpT protease. It is acknowledged that the specification describes cleavage of the motif LRLYR↓[X]HHGS, wherein X is specific for some cleavage by the encompassed OmpT variants. However, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 13 and 16 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, having a P1 Arg or Lys and not having a P1' Arg or Lys, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, E, or H at D^{97} of OmpT protease.

It is acknowledged that the specification describes cleavage of the motif LRLYR↓[X]HHGS, wherein X is specific for some cleavage by the encompassed OmpT variants. However, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 14, 17, 21, and 22 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, having a P1 Arg or Lys, not having a P1' Arg or Lys, and having a single basic amino acid or two or three consecutive basic amino acids situated at any site in the amino acid sequence from P10-P3 position or P3'-P5', using an OmpT protease having any substitution of A, L, F, M, S, T, C, Q, N, E, or H at D^{97} of OmpT protease. It is acknowledged that the specification describes cleavage of the motif LRLYR↓[X]HHGS, wherein X is specific for some cleavage by the encompassed OmpT variants. However, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease

variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 19 and 20 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site in the amino acid sequence having two or three consecutive basic amino acids between P10-P3, using an OmpT protease having any substitution of A, L, F, M, S, T, C, Q, N, E, or H at D^{97} of OmpT protease. The specification teaches only one representative species of such methods using L^{97} (Fig11-12) and three representative species of such methods using M^{97} (Fig5,15,16). The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. In addition, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative

species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 25 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, comprising R-R-R-A-R(P1)↓X(P1') wherein P1' is any amino acid, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N, Q, E, or H replacing D^{97} of OmpT protease. The specification teaches only one representative species of such methods using L^{97} (Fig11-12) and three representative species of such methods using M^{97} (Fig5,15,16). The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Ala, Val, Ile, Phe, Tyr, and Trp, like Met and Leu, are considered to be neutral/hydrophobic amino acids. Based on said knowledge in the art, the skilled artisan would believe that, more likely than not, the OmpT A^{97}, V^{97}, I^{97}, F^{97}, and Y^{97} variants would cleave the same motifs cleaved by the OmpT M^{97} and L^{97} variants. However, the skilled would not believe that, more likely than not, the variants having S, T, C, N, Q, E, or H replacing D^{97} of OmpT protease would cleave the motifs cleaved by the OmpT M^{97} and L^{97} variants. Moreover, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit

cleavage by any of the encompassed OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 26 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, comprising D-A-R-R-R-A-R(P1)↓X(P1') wherein P1' is any amino acid, using an OmpT protease having any substitution of A, L, F, M, S, T, C, N,Q, E, or H replacing D^{97} of OmpT protease. The specification teaches only one representative species of such methods using M^{97} (Fig15,16). The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Ala, Val, Ile, Leu, Phe, Tyr, and Trp, like Met, are considered to be neutral/hydrophobic amino acids. Based on said knowledge in the art, the skilled artisan would believe, that more likely than not the OmpT A^{97}, V^{97}, I^{97}, L^{97}, F^{97}, and Y^{97} variants would cleave the same motifs cleaved by the OmpT M^{97} variant. However, the skilled would not believe that, more likely than not, the variants having S,

T, C, N, Q, E, or H replacing D^{97} of OmpT protease would cleave the motifs cleaved by the OmpT M^{97} variant. Moreover, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by any of the encompassed OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 27 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site using an OmpT protease having any substitution of L, M, or H at D^{97} of OmpT protease. It is acknowledged that the specification describes cleavage of the motif LRLYR↓[X]HHGS, wherein X is specific for some cleavage by the encompassed OmpT variants. However, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of

representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 28 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, having a P1' Ser or Ala, using an OmpT protease having a substitution of Leu at D^{97} of OmpT protease. The specification teaches only six representative species of such methods using L^{97} (Fig11-12; Exmp13;Table 1). It is noted that the species disclosed in Example 13 (Table 1) represent only a single sequence for the context of the variant P1' residue. Thus, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants, such the skilled artisan would recognize applicants were in possession of the recited genus of methods at the time of filing. The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in

such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 29 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, having a P1' F, A, S, C or Y, using an OmpT protease having a substitution of M at D^{97} of OmpT protease. The specification teaches only five representative species of such methods using M^{97} (Fig5,15; Exmp13;Table 1). It is noted that the species disclosed in Example 13 (Table 1) represent only a single sequence for the context of the variant P1' residue. Thus, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants, such the skilled artisan would recognize applicants were in possession of the recited genus of methods at the time of filing. The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 30 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. These claims are directed to a genus of methods for cleaving a polypeptide at any site, having a P1' A, V, I, M, S, T, C, or N, using an OmpT protease having a substitution of H at D^{97} of OmpT protease. The specification teaches only eight representative species of such methods using H^{97} (Fig11-12; Exmp13;Table 1). It is noted that the species disclosed in Example 13 (Table 1) represent only a single sequence for the context of the variant P1' residue. Thus, the specification fails to describe the amino acids at positions P10-P2 and P2'-P5', or combinations thereof, that enhance cleavage, are permissive for cleavage, or inhibit cleavage by the OmpT protease variants, such the skilled artisan would recognize applicants were in possession of the recited genus of methods at the time of filing. The specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a polypeptide by a variant OmpT protease. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 32 is so broad as to encompass cleaving a protein comprising any target protein having any structure and having the function any calcitonin precursor. The specification teaches

only a one representative species of such methods, wherein the peptide having calcitonin precursor activity has the sequence CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAPG (Fig11). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a fusion protein comprising any peptide, having any sequence and having calcitonin precursor activity, with OmpT protease. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Regarding the prior rejection of Claims 12, 15-16, 23, and 26-27 under 35 USC 112, first paragraph/written description, Applicants traverse with the following arguments, which are relevant to the rejections above.

(A) The Federal Circuit explicitly holds that working examples covering the full scope of the claims are not required for an adequate written description. The Office's requirement for specific examples for each and every variant encompassed by the claim explicitly contradicts this holding.

(B) Relevant here is whether the disclosed species are representative of the claimed genus, such that the skilled artisan would recognize that Applicants possessed the "necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed."

(C) The Specification describes that (1) OmpT protease and other OmpT-like proteases from Salmonella, Yersinia, and Shigella are known to be members of the omptin family, which

has been well characterized; (2) these proteases share significant amino acid sequence identity; (3) the crystal structure of OmpT has been published, providing a well-characterized relationship between structure and function; (4) the interaction between OmpT's active site with its corresponding substrates has been studied in detail; and (5) there is standard knowledge in the field regarding the generation of variant proteases and various substrate peptides. See e.g., lines 26-33 on page 1 and the paragraph bridging pages 27-28 of the Specification; see also Kramer supra; see also Vandeputte-Rutten et al., 20 EMBO J. 5033 (2001), and Stathopoulos, 12 MEMBR. CELL BIOL. 1 (1998).

(A) Reply: It is acknowledged that working examples covering the full scope of the claims are not required for an adequate written description. MPEP 2163II3(a) states:

> "Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. See, e.g., *Eli Lilly*."

As explained above, the disclosed examples fail to provide a description of the necessary common attributes or features of amino acid residues at positions P10-P2 and P2'-P5', and combinations thereof, that are favored, permitted, or non-favored for cleavage by each encompassed OmpT protease Asp97 variant.

(B) Reply: See (A), above.

(C) Reply: It is acknowledged that the crystal structure of OmpT protease was published by Vandeputte-Rutten et al, 2001; however, Vandeputte-Rutten et al fails to teach any Asp^{97} OmpT protease variants or substrates thereof. Only the OmpT protease Asp^{97}Ala variant, and one substrate thereof, were known at the time of filing (Kramer et al, 2001). However, the

results of Kramer et al do not agree with the results of the instant application, which shows that the OmpT protease Asp^{97}Ala variant does not cleave -Arg↓Arg-. As explained above, under 35 USC 112, first paragraph/enablement and second paragraph, the substrate specificity for the encompassed E. coli OmpT protease variants, encompassing up to positions P10-P5', remained unpredictable.

It is acknowledged that other OmpT-like proteases have 40-73% homology with OmpT protease (Hritonenko et al, 2007; Table I). However, said homologies fail to provide evidence as to the substrate specificity for variants of OmpT protease with a substitution at Asp^{97}. It is acknowledged that teachings describing the substrate specificity of OmpT-like proteases with a substitution at Asp^{97} might be applicable to OmpT protease with a substitution at Asp^{97}. However, the specification fails to point to such teachings. Moreover, the art teaches that E. coli OmpP protease, which has highest identity with OmpT protease, has a different substrate specificity (Hwang et al, 2007). Thus, the art teaches away from using other omptin proteases as models for the substrate specificity of Asp^{97} OmpT protease variants.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Okuno et al, 2002b (Biotechnol Appl Biochem. 2002 Oct;36(Pt 2):77-84[on-line availability 05-AUG-2002]; IDS of 28-MAR-2006). Okuno et al, 2002b teaches cleaving the peptide ELELYK↓RHHG with OmpT

protease (Table 3). Therefore, Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Okuno et al, 2002b (Biotechnol Appl Biochem. 2002 Oct;36(Pt 2):77-84).

In their remarks of September 14, 2009 (pg 11-13), Applicants traversed the Offices' assertion, in the Restriction Requirement of January 9, 2009, that Okuno et al, 2002b anticipates Claim 1. Applicants argued that Okuno et al, 2002b fails to cleave a motif having a single basic amino acid within P10-P3 or P3'-P5', wherein said single basic amino acid is not at P6 or P4. Applicants further argued that the genus of basic amino acids consists of Arg and Lys. Said arguments are not found to be persuasive for the following reasons. First, Okuno et al, 2002b teaches cleaving the peptide ELELYK↓RHHG (having a single basic His at P3') with OmpT protease (Table 3). Second, the skilled artisan would have known that histidine is a basic amino acid (see definition enclosed). Moreover, original Claim 6 recites "wherein the basic amino acids are arginine and/or lysine"; thus, original parent Claim 1 encompassed more than just Arg and Lys. The skilled artisan would have assumed that Claim 1 encompassed histidine.

Claims 1, 6, and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Sugimura et al, 1988b. Sugimura et al teaches cleavage of Dynorphin A at the motif Tyr-Gly-Gly-Phe-Leu-Arg↓Arg-Ile-Arg-Pro-Lys-Leu-Lys, which has a P1 Arg and a P3' Arg (Table 1). Therefore, Claims 1, 6, and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Sugimura et al, 1988b.

Claims 1 and 34 are rejected under 35 U.S.C. 102(b) as being anticipated by Okuno et al, 2002a (Biosci Biotechnol Biochem. 2002 Jan;66(1):127-34; IDS of 28-MAR-06). Okuno et al teaches cleavage of several fusion proteins using bacteria-derived OmpT protease, wherein the

cleavage motif comprises a P1 Arg and a P3' histidine (Table 1). Therefore, Claims 1 and 34 are rejected under 35 U.S.C. 102(b) as being anticipated by Okuno et al, 2002a.

Claims 1, 34, and 35 rejected under 35 U.S.C. 102(b) as being anticipated by Yabuta et al, 1995. Yabuta et al teach cleaving a recombinantly expressed polypeptide in cells expressing OmpT protease, wherein the cleavage site comprises a P1 Arg, a P1' Arg, and two consecutive basic amino acids within P3' to P5' (Fig2). Therefore, Claims 1, 34, and 35 rejected under 35 U.S.C. 102(b) as being anticipated by Yabuta et al, 1995.

Claim 12 is rejected under 35 U.S.C. 102(b) as being anticipated by Kramer et al, 2001 (IDS 28-MAR-06). Kramer et al teaches an OmpT protease variant having an $Asp^{97}Ala$ substitution and use of said variant to cleave at Ala-Arg↓Arg-Ala (Fig2). Therefore, Claim 12 is rejected under 35 U.S.C. 102(b) as being anticipated by Kramer et al, 2001.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 2 and 31-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yamamoto et al, 1996 in view of Dekker et al, 2001 (IDS 28-MAR-06). Yamamoto et al teaches making a fusion protein comprising the target protein motilin (FVPIFTYGELQRMQEKERNKGQ) in E. coli host cells (Col 4-Formula 1a (A-B-C); Col 7, parg2; Example 1) and cleaving said fusion protein, at a linker motif comprising Lys↓Arg, using OmpT protease (Col 6, parg5). Yamamoto et al does not teach cleaving a fusion protein wherein

the P1 residue is an Arg in the fusion partner and the P1' residue is the N-terminal Phe of motilin. Dekker et al teaches that OmpT protease cleaves at the motif Arg↓Phe-Val (Table 2). It would have been obvious to a person of ordinary skill in the art to adapt the method of Yamamoto et al such that OmpT protease is used to cleave a fusion protein, wherein the P1 residue is an Arg in the fusion partner and the P1' residue is the N-terminal Phe of motilin, as set forth by R↓FVPIFTYGELQRMQEKERNKGQ. Motivation to do so is provide by the desire to isolate motilin without additional amino acids. The expectation of success is high, as Dekker et al teaches that Phe at P1'is allowed and Val at P2' is preferred (Table 2). Therefore, Claims 2 and 31-33 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yamamoto et al, 1996 in view of Dekker et al, 2001.

Claims 3, 8, and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2002a, Okuno et al, 2002b, or Sugimura et al, 1988a in view of Dekker et al, 2001 and Okuno et al, 2002b. As described above, Okuno et al, 2002a, Okuno et al, 2002b, and Sugimura et al teach cleavage of several polypeptides by OmpT protease. Okuno et al, 2002a, Okuno et al, 2002b, and Sugimura et al do not teach converting an undesirable OmpT cleavage site to a non-cleaved site by setting an acidic amino acid at the P3 position of the undesirable OmpT cleavage site. Dekker et al teaches that the acidic amino acid Asp or Glu at the P2 position inhibits cleavage by OmpT protease (Table 2). Okuno et al, 2002b teaches that the acidic amino acid Asp or Glu at the P4 position inhibits cleavage by OmpT protease (Table 2). It would have been obvious to a person of ordinary skill in the art to convert any undesirable OmpT cleavage sites, in the fusion protein of Okuno et al, 2002a or the protein of Sugimura et al, to non-cleaved sites by setting Asp or Glu at the P3 position of the undesirable OmpT cleavage site. Motivation to do

so is provide by the desire to avoid cleavage at the undesirable sites. The expectation of success is high, as based on Okuno et al, 2002b and Dekker et al, the skilled artisan would have believed that, more likely than not, an acidic amino acid at the P3 position would inhibit cleavage by OmpT protease. Therefore, Claims 3, 8, and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2002a, Okuno et al, 2002b, or Sugimura et al, 1998 in view of Dekker et al, 2001 and Okuno et al, 2002b.

Claims 4, 5, and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stumpe et al, 1998 in view of Suzuki et al, 1972 and Sugimura et al, 1988a. Stumpe et al teaches that protamines are cleaved by OmpT protease (Fig2). Stumpe et al does not specifically teach cleavage of a protamine comprising the peptide of SEQ ID NO: 11. Suzuki et al teaches that the protamine component Clupein YII comprises the sequence ^{23}Arg-Arg-Arg-Ala-Arg-Arg^{28} (Fig7), wherein residues 23-27 represent SEQ ID NO: 11. It would have been obvious to a person of ordinary skill in the art to use OmpT protease to cleave the specific protamine component Clupein YII between ^{27}Arg↓Arg^{28}, wherein positions P1-P5 are as set forth by SEQ ID NO: 11. Motivation to do so is provide by the desire to demonstrate that OmpT protease cleaves the specific protamine component Clupein YII. The expectation of success is high, as Stumpe et al teaches that it "was not unexpected that OmpT is the extracytoplasmic protease that inactivates the highly cationic peptide protamine (pg4006, parg3) and it was known in the art that E. coli OmpT protease can cleave between the dibasic residues –Ala-Arg↓Arg (Kramer et al). Therefore, Claims 4, 5, and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stumpe et al, 1998 in view of Suzuki et al, 1972 and Sugimura et al, 1988a.

Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, or Dekker et al, 2001 in view of Grodberg et al, 1988a. As described above, Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al teach cleavage of a series of polypeptides by OmpT protease. Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al do not teach using a bacterial cell expressing OmpT protease as a source for cleavage activity. Grodberg et al teaches using a bacterial cell, expressing OmpT protease on the surface, as a source for cleavage activity (pg1247, parg3; Fig1/W3110). It would have been obvious to a person of ordinary skill in the art to use the bacterial cell of Grodberg et al as a source for cleaving the polypeptides of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al. Motivation to do so is provide by the desire to cleave said proteins without the effort of purifying the OmpT protease. The expectation of success is high, as all methods were known in the art. Therefore, Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, or Dekker et al, 2001 in view of Grodberg et al, 1988a.

Claims 2, 31, 33, and 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, 2001 in view of Yabuta et al, 1995. As described above, Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al teach cleavage of a series of polypeptides by OmpT protease. Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al do not teach recombinantly expressing their proteins in a cell expressing OmpT protease as a means to cleave the proteins. Yabuta et al teaches recombinantly expressing a fusion protein, comprising an OmpT cleavage site, in an E. coli host cell expressing OmpT protease as a means to cleave the fusion protein (Fig4). It would

have been obvious to a person of ordinary skill in the art to use the method of Yabuta et al to express the proteins of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al as fusion proteins, in the host cell expressing OmpT protease, as a means to cleave said proteins by OmpT protease. Motivation to do so is provide by the desire to cleave said proteins without the effort of purifying the OmpT protease. The expectation of success is high, as all methods were known in the art. Therefore, Claims 2, 31, 33, and 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, 2001 in view of Yabuta et al, 1995.

Claims 12-14, 21, and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, 2001 in view of Kramer et al, 2001. As described above, Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al teach cleavage of a series of polypeptides by OmpT protease. Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al do not teach do not teach cleaving said proteins with an OmpT protease variant having a substitution at position Asp^{97}. Kramer et al teaches an OmpT protease variant having an $Asp^{97}Ala$ substitution and that said variant cleaves at Ala-Arg↓Arg-Ala (Fig2). It would have been obvious to a person of ordinary skill in the art to determine which of the proteins of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al are cleaved by the OmpT protease $Asp^{97}Ala$ variant. Motivation to do so is provide by the desire to examine the cleavage motif requirement of said variant by using the sequences of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the OmpT protease $Asp^{97}Ala$ variant of Kramer et al would cleave one or more of the numerous substrates

taught by Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, including those having a P1' that is, or is not, Arg or Lys and/or a single Arg at either P10-P3 or P3'-P5'. Therefore, Claims 12-14, 21, and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, 2001 in view of Kramer et al, 2001.

Claims 12-14, 21, and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, and Kramer et al renders obvious cleavage of one or more OmpT substrates with the $Asp^{97}Ala$ OmpT protease variant of Kramer et al. Said combination does not teach cleavage of one or more OmpT protease substrates with an OmpT protease variant having a substitution at Asp^{97} with a residue other than Ala. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Asp and Glu are considered to both be acidic amino acids. Kramer et al teaches that Asp^{97} aids in substrate binding by coordinating with the P1' Arg of known OmpT protease -Arg↓Arg- substrates (Fig 4). It would have been obvious to the skilled artisan to make the Glu^{97} OmpT protease variant (conservative substitutions for Asp) and use the variant to cleave the OmpT protease substrates of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the Glu^{97} OmpT protease has essentially the same substrate specificity as the parent Asp^{97} OmpT protease. Therefore, Claims 12-14, 21, and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et

al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Claims 12-14, and 27-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, and Kramer et al renders obvious cleavage of one or more OmpT substrates with the $Asp^{97}Ala$ OmpT protease variant of Kramer et al. Said combination does not teach cleavage of one or more OmpT protease substrates with an OmpT protease variant having a substitution at Asp^{97} with a residue other than Ala. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, (i) Ala, Val, Ile, Leu, Phe, Tyr, Trp, and Met are considered to be neutral/hydrophobic amino acids and (ii) Gly, Ser, Thr, Cys, Asn, and Gln are considered to be small hydrophobic/polar amino acids. It would have been obvious to a person of ordinary skill in the art to make OmpT protease variants having a substitution at Asp^{97} with any of (i) Ala, Val, Ile, Leu, Phe, Tyr, Trp, and Met or (ii) Ser, Thr, Cys, Asn, and Gln and use said variants to cleave the substrates of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, including those having a single basic amino acid at either P10-P3 or P3'-P5' and/or a P1' that is, or is not, Arg or Lys. Motivation to do so is provided by the desire to cleave OmpT protease substrates. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, (i) the OmpT protease variants having a substitution at Asp^{97} with any of Ala, Val, Ile, Leu, Phe, Tyr, Trp, and Met would association with a substrate comprising a P1' I, F, V, A, Y, M, W, or L and (ii) the OmpT protease variants

having a substitution at Asp^{97} with any of Gly, Ser, Thr, Cys, Asn, and Gln would have enhance association with a substrate comprising a P1' S, C, N, Q, T, or G. This expectation of success is based on the fact that (i) neutral/hydrophobic amino acids are more likely to associate with each other, (ii) small hydrophobic/polar amino acids are more likely to associate with each other, and (iii) substrate binding is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 12-14, and 27-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Claims 12-14, 21, and 22, 27 and, 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Sugimura et al, 2002a, Okuno et al, 2002b, Dekker et al, and Kramer et al renders obvious cleavage of one or more OmpT substrates with the Asp^{97}Ala OmpT protease variant of Kramer et al. Said combination does not teach cleavage of one or more OmpT protease substrates with an OmpT protease variant having a substitution at Asp^{97} with a residue other than Ala. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Asp and Glu are considered to both be acidic amino acids, while Arg, Lys, and His are considered to be basic amino acids. Kramer et al teaches that Asp^{97} aids in substrate binding by coordinating with the P1' Arg of known OmpT protease -Arg↓Arg- substrates (Fig 4). It would have been obvious to the skilled artisan to make the Arg^{97}, Lys^{97}, and His^{97} OmpT protease variants and use the variants to cleave OmpT protease

substrates of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al having a substitution of the P1' position with an acidic amino acid. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the Arg^{97}, Lys^{97}, and His^{97} OmpT proteases would have substrate specificity for a P1' acidic amino acid. This expectation of success is based on the fact that (i) the Arg^{97}, Lys^{97}, and His^{97} basic amino acids of the OmpT protease variants are more likely to associate with a P1' acidic amino acid and (ii) substrate binding is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 12-14, 21, and 22, 27 and, 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Claims 15-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Yabuta et al, 1995. As described above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al and Kramer et al teaches cleavage of OmpT protease substrates with the OmpT protease Asp^{97}Ala variant. Said combination does not teach transforming host cells with a plasmid encoding a fusion protein comprising a cleavage site for an Asp^{97}Ala variant OmpT protease. Yabuta et al teaches transforming E. coli host cells with a plasmid encoding a fusion protein comprising a cleavage site for OmpT protease and cleaving of said fusion protein with the endogenous OmpT protease. It would have been obvious to a person of ordinary skill in the art to combine the teachings of the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, and

Kramer et al with Yabuta et al to make a method wherein fusion proteins comprising the substrates of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, are co-expressed in a host cell with the OmpT $Asp^{97}Ala$ variant taught by Kramer et al. Motivation to do so is provide by the desire to cleave said substrates without the effort of isolating the OmpT $Asp^{97}Ala$ variant. The expectation of success is high, as all methods are well known in the art and the skilled artisan would expect that, more likely than not, one or more of the substrates of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al would be cleaved by the OmpT $Asp^{97}Ala$ variant. Therefore, Claims 15-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Yabuta et al, 1995.

Claims 15-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Yabuta et al, 1995. As explained above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler teaches cleavage of OmpT protease substrates with the $Asp^{97}Glu$ OmpT protease variant. Said combination does not teach transforming host cells with a plasmid encoding a fusion protein comprising a cleavage site for the $Asp^{97}Glu$ variant OmpT protease. Yabuta et al teaches transforming E. coli host cells with a plasmid encoding a fusion protein comprising a cleavage site for OmpT protease and cleaving of said fusion protein with the endogenous OmpT protease. It would have been obvious to a person of ordinary skill in the art to combine the teachings of the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler with Yabuta et al to make a method wherein

fusion proteins comprising the substrates of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al are co-expressed in a host cell with the OmpT $Asp^{97}Glu$ variant taught by the combination of Kramer et al and Metzler. Motivation to do so is provided by the desire to cleave said substrates without the effort of isolating the OmpT $Asp^{97}Glu$ variant. The expectation of success is high, as all methods are well known in the art and the skilled artisan would expect that the OmpT $Asp^{97}Glu$ variant has essentially the same substrate specificity as OmpT protease. Therefore, Claims 15-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Yabuta et al, 1995.

Claims 18, 23, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of the combination of Dekker et al, 2001 and Okuno et al, 2002b. As explained above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler teaches cleavage of OmpT protease substrates with the $Asp^{97}Glu$ OmpT protease variant. Said combination does not teach converting an undesirable $Asp^{97}Glu$ OmpT protease variant cleavage site to a non-cleaved site by setting an acidic amino acid at the P3 position of the undesirable cleavage site. Dekker et al teaches that the acidic amino acid Asp or Glu at the P2 position inhibits cleavage by OmpT protease (Table 2). Okuno et al, 2002b teaches that the acidic amino acid Asp or Glu at the P4 position inhibits cleavage by OmpT protease (Table 2). It would have been obvious to a person of ordinary skill in the art to convert any undesirable cleavage sites, in a polypeptide substrate cleaved by the $Asp^{97}Glu$ OmpT protease variant, by setting the P3 position of any said

undesirable cleavage site to Asp or Glu. Motivation to do so is provide by the desire to avoid cleavage at undesirable sites. The expectation of success is high, as (i) the skilled artisan would have believe that the Glu^{97} OmpT protease has essentially the same substrate specificity as the parent Asp^{97} OmpT protease and (ii) based on Okuno et al, 2002b and Dekker et al, the skilled artisan would have believed that an acidic amino acid at the P3 position would inhibit cleavage by the Asp^{97}Glu OmpT protease. Therefore, Claims 18, 23, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of the combination of Dekker et al, 2001 and Okuno et al, 2002b.

Claims 19, 20, and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of the combination of Stumpe et al, 1998 and Suzuki et al, 1997. As explained above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler teaches cleavage of OmpT protease substrates with the Asp^{97}Glu OmpT protease variant. Said combination does not teach cleaving a substrate comprising SEQ ID NO: 11. As explained above, the combination of Stumpe et al and Suzuki et al teaches using OmpT protease to cleave the specific protamine component Clupein YII between $^{27}Arg{\downarrow}Arg^{28}$, wherein positions P1-P5 are as set forth by SEQ ID NO: 11. It would have been obvious to a person of ordinary skill in the art to use the Asp^{97}Glu OmpT protease variant to cleave the specific protamine component Clupein YII between $^{27}Arg{\downarrow}Arg^{28}$, wherein positions P1-P5 are as set forth by SEQ ID NO: 11. Motivation to do so is provide by the desire to cleave the specific protamine component Clupein YII. The

expectation of success is high, the skilled artisan would have believed that the Glu^{97} OmpT protease has the same substrate specificity as the parent Asp^{97} OmpT protease. Therefore, Claims 19, 20, and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of the combination of Stumpe et al, 1998 and Suzuki et al, 1997.

Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Grodberg et al, 1988. As described above, the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, and Kramer et al renders obvious cleavage of one or more OmpT substrates with the Asp^{97}Ala OmpT protease variant of Kramer et al. Said combination does not teach using a bacterial cell expressing the Asp^{97}Ala OmpT protease variant of Kramer et al as a source for cleavage activity. Grodberg et al teaches using a bacterial cell expressing the parent OmpT protease on the surface as a source for cleavage activity (pg1247, parg3; Fig1/W3110). It would have been obvious to a person of ordinary skill in the art to make a bacterial cell expressing the Asp^{97}Ala OmpT protease variant of Kramer et al and use said bacterial cell as a source for cleaving the proteins of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, or Dekker et al. Motivation to do so is provide by the desire to cleave said proteins without the effort of purifying the variant OmpT protease. The expectation of success is high, as all methods were known in the art and Kramer et al teaches that their Asp^{97}Ala OmpT protease variant cleaves at Arg-Arg (Fig2). Therefore, Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a,

Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Grodberg et al, 1988.

Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Grodberg et al, 1988. As explained above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler teaches cleavage of OmpT protease substrates with the $Asp^{97}Glu$ OmpT protease variant. Said combination does not teach using a bacterial cell expressing the $Asp^{97}Glu$ OmpT protease rendered obvious by Kramer et al and Metzler as a source for cleavage activity. Grodberg et al teaches using a bacterial cell expressing the parent OmpT protease on the surface as a source for cleavage activity (pg1247, parg3; Fig1/W3110). It would have been obvious to a person of ordinary skill in the art to make a bacterial cell expressing the $Asp^{97}Glu$ OmpT protease variant and use said bacterial cell as a source for cleaving the proteins of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, or Dekker et al. Motivation to do so is provide by the desire to cleave said proteins without the effort of purifying the variant OmpT protease. The expectation of success is high, as all methods were known in the art and the skilled artisan would believe that the $Asp^{97}Glu$ OmpT protease variant has essentially the same substrate specificity as OmpT protease. Therefore, Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Grodberg et al, 1988.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Kramer et al, 2001 in

view of Yabuta et al, 1995. As described above, the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, and Kramer et al renders obvious cleavage of one or more OmpT substrates with the Asp^{97}Ala OmpT protease variant of Kramer et al. Said combination does not teach recombinantly expressing their polypeptides in a cell expressing the Asp^{97}Ala OmpT protease variant as a means to cleave the polypeptides of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al. Yabuta et al teaches recombinantly expressing a protein, comprising an OmpT cleavage site, in a cell expressing OmpT protease as a means to cleave the protein (Fig4). It would have been obvious to a person of ordinary skill in the art to use the method of Yabuta et al to express the polypeptides of Sugimura et al, Okuno et al, 2002a, and Okuno et al, 2002b in a cell expressing the Asp^{97}Ala OmpT protease variant of Kramer et al as a means to cleave said polypeptides by said OmpT protease variant. Motivation to do so is provide by the desire to cleave said polypeptides without the effort of purifying the OmpT protease variant. The expectation of success is high, as all methods were known in the art and Kramer et al teaches that their Asp^{97}Ala OmpT protease variant cleaves at Arg-Arg (Fig2). Therefore, Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Kramer et al, 2001 in view of Yabuta et al, 1995.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Yabuta et al, 1995. As explained above, the combination of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, Kramer et al, 2001 and Metzler teaches cleavage of OmpT protease substrates with the Asp^{97}Glu OmpT protease

variant. Said combination does not teach recombinantly expressing the polypeptides of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al in a cell expressing the $Asp^{97}Glu$ OmpT protease variant as a means to cleave the polypeptides. Yabuta et al teaches recombinantly expressing a protein, comprising an OmpT cleavage site in a cell expressing OmpT protease as a means to cleave the protein (Fig4). It would have been obvious to a person of ordinary skill in the art to use the method of Yabuta et al to express the polypeptides of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al in a cell expressing the $Asp^{97}Glu$ OmpT protease variant as a means to cleave said polypeptides by said OmpT protease variant. Motivation to do so is provide by the desire to cleave said polypeptides without the effort of purifying the $Asp^{97}Glu$ OmpT protease variant. The expectation of success is high, as all methods were known in the art and the skilled artisan would believe that the $Asp^{97}Glu$ OmpT protease variant has essentially the same substrate specificity as OmpT protease. Therefore, Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, Kramer et al, 2001, and Metzler, 2001 in view of Yabuta et al, 1995.

Allowable Subject Matter

No claims are allowable.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate

pages. It is also requested that the serial number of the application be referenced on every page of the response.

It is also requested that Applicants identify support, within the original application, for any amendments to the claims and specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sheridan L. Swope whose telephone number is 571-272-0943. The examiner can normally be reached on M-F; 9:30-7 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang, can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/SHERIDAN SWOPE/
Primary Examiner, Art Unit 1652

/Andrew Wang/
Supervisory Patent Examiner, Art Unit 1652